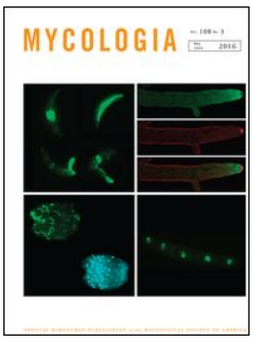

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Diversity and distribution patterns of root-associated fungi on herbaceous plants in alpine meadows of southwestern China

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Abstract: The diversity of root-associated fungi associated with four ectomycorrhizal herbaceous species, *Kobresia capillifolia*, *Carex parva*, *Polygonum macrophyllum* and *Potentilla fallens*, collected in three sites of alpine meadows in southwestern China, was estimated based on internal transcribed spacer (ITS) rDNA sequence analysis of root tips. Three hundred seventy-seven fungal sequences sorted to 154 operational taxonomical units (sequence similarity of ≥ 97% across the ITS) were obtained from the four plant species across all three sites. Similar taxa (in GenBank with ≥ 97% similarity) were not found in GenBank and/or UNITE for most of the OTUs. Ectomycorrhiz a made up 64% of the fungi operational taxonomic units (OTUs), endophytes constituted 4% and the other 33% were unidentified root-associated fungi. Fungal OTUs were represented by 57% basidiomycetes and 43% ascomycetes. *Inocybe*, *Tomentella/Thelophora*, *Sebacina*, *Hebeloma*, Pezizomycotina, *Cenococcum geophilum* complex, *Cortinarius*, *Lactarius* and Helotiales were OTU-rich fungal lineages. Across the sites and host species the root-associated fungal communities generally exhibited low host and site specificity but high host and sampling site preference. Collectively our study revealed noteworthy diversity and endemism of root-associated fungi of alpine plants in this global biodiversity hotspot.

Key words: alpine areas, herbaceous plants, ITS rDNA, root-associated fungi

Introduction

Alpine areas cover about 4% of the Earth's surface and are characterized by environments with low temperatures, low atmospheric pressure, short growing periods and slow decomposition (Körner 2003). In these areas, where the availability of important nutrients is restricted by environmental conditions (Körner 1999), plants have developed morphological, anatomical and/or physiological adaptations, including mycorrhizal associations and physical modifications of root structure (Berendse and Jonasson 1992, Muthukumar et al. 2004). The formation of fungal symbioses is thought to be an effective way of improving alpine plant nutrition (Haselwandter 1987, Gardes and Dahlberg 1996, Treu et al. 1996, Kagawa et al. 2006, Kytöviita and Ruotsalainen 2007) and dauciform roots, which are carrot-shaped lateral roots with a smooth, colored surface free of root hairs, also have been shown to enhance the efficiency of nutrient acquisition of plants growing in stressed environments (Miller 2005, Shane et al. 2006).

Investigations regarding the mycorrhizal status of plants in the genera *Kobresia*, *Carex*, *Polygonum* and *Potentilla* have revealed that ectomycorrhizal associations are common (Harley and Harley 1987, Gardes and Dahlberg 1996, Massicotte et al. 1998, Cripps and Eddington 2005, Gai et al. 2006, Kagawa et al. 2006, Mühlmann and Peintner 2008, Mühlmann et al. 2008, Gao and Yang 2010, Li et al. 2014). Ascomycete fungi also have been detected on ectomycorrhizal root tips of alpine plants (Bjorbækmo et al. 2010, Blaalid et al. 2012, Yao et al. 2013), including *Kobresia myosuroides* and *Polygonum viviparum* (Schadt and Schmidt 2001, Ammarellou and Saremi 2008, Mühlmann et al. 2008, Mühlmann and Peintner 2008), indicating multiple type of fungal colonization often are present (Harley and Harley 1987, Wagg and Pautler 2008, Tedersoo et al. 2009, Becerra et al. 2009, Gao and Yang 2010). Along with colonization of normal fine roots, dauciform roots present on *Kobresia* spp. and *Carex* sp. have been also shown to be colonized by ectomycorrhiza and/or other mycobionts (Gao and Yang 2010). However, there is currently no relevant knowledge about the belowground fungal associations of *Kobresia capillifolia*, *Carex parva*, *Polygonum macrophyllum* and *Potentilla fallens* and those of the dauciform roots of *Kobresia capillifolia* and *Carex parva*.

Alpine meadows, which comprise about 16% of the grassland area of the Tibetan Plateau, are a significant component of the Hengduan Mountains in the easternmost Himalaya in southwestern China (Gai et al. 2009), one of the world's 25 biodiversity hotspots. A large number of exclusive and valuable plant and fungi species grow in the region (Wu and Zhu 1987), and the symbiosis between plants and fungi may be

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crucial for their survival in the harsh environment of this alpine area. However, there have been few studies on root-associated fungi of herbaceous plants in the region. In addition, because alpine fungal communities are species rich and many of the species are unique to the Hengduan Mountains (Gao 2009, Gao and Yang 2010), a study of the diversity of root-associated fungi associated with plants in the region can provide a basis for biodiversity research, ecological studies, conservation efforts and resource exploitation in the southwestern mountains of China.

In this study three typical alpine meadows in the Hengduan Mountains were selected and four of the dominant herbaceous plant species in the region, *Kobresia capillifolia*, *Carex parva*, *Polygonum macrophyllum* and *Potentilla fallens* were chosen with the aim of identifing the diversity of root-associated fungi species and examine the distribution of mycobiont assemblages in terms of host-plant species and sampling sites.

MATERIALS AND METHODS

Sampling and sample processing.—The sampling sites, which are in the Hengduan Mountain range in the eastern Himalaya of southwestern China, consist of three alpine meadows in the Hong Mountains (4500 m, 27°50′N, 99°24′E), Daxue Mountains (4250 m, 27°19′N, 100°06′E) and Baimaxue Mountains (4200 m, 27°33′N, 99°32′E) in Shangri-La and Deqin counties of Yunnan province. The sampling areas experience annual mean temperatures of 0 C, −0.4 C and −1.0 C and annual mean precipitation of 800–850 mm, 750–800 mm and 600–650 mm (Duan 1997, Li 1997), respectively. The plant communities are grasslands, in which *Kobresia* spp., *Carex* spp., *Polygonum* spp. and *Potentilla* spp. are widely distributed (Wu and Zhu 1987). Sampling of *Kobresia capillifolia*, *Carex parva*, *Polygonum macrophyllum* and *Potentilla fallens* was carried out in mid-Aug 2008. Five samples 5–6 m apart were randomly collected for each plant species within a 50 × 50 m^2 square at each sampling site, resulting in a total of 60 plant samples from the four plant species. Plants, including their roots and aboveground parts, and surrounding soil were excavated, resulting in plots about 30 × 20 × 20 cm (long, wide, deep) each. Plant samples were stored for further treatment in the original soil at 4 C for a maximum of 2 wk.

Approximately 100 ectomycorrhizal root tips were haphazardly selected from root systems of each plant sample, resulting in a total of 6000 root tips from the 60 plant samples. Root tips were examined under a dissecting microscope at 3× magnification, cleaned of any debris, and macroscopically assigned to morphotypes based on color, mantle surface, ramification pattern and occurrence of emanating hyphae (Agerer 2006). Dauciform roots on *Kobresia capillifolia* and *Carex parva* were examined and sorted by color. Three–five representative root tips of each morphotype were stored in a saturated NaCl/CTAB solution at −20 C for molecular identification.

PCR and sequence analyses of the internal transcribed spacer (ITS) rDNA region.—DNA extraction from root tips followed the procedures of Hibbett and Vilgalys (1993) with modifications. Primer combinations of ITS1F × ITS4 (Gardes and Bruns 1993), ITS1F × LB-W and ITS1F × LA-W (Tedersoo et al. 2008) were used to amplify the rDNA ITS region. Amplified products were purified and ligated into TaKaRa pMD18-T plasmids (TaKaRa Bio Inc., Shiga, Japan), which were cloned into *Escherichia coli* strain DH5a. Ten clones were selected randomly for each amplified product and automatically sequenced with an ABI 3730xl DNA Analyzer (Applied Biosystems). Sequences were deposited in GenBank with accession numbers JQ346810–JQ347225.

Sequences from each of the representative root tips were sorted into operational taxonomical units (OTUs) at a sequence similarity of ≥ 97% across the ITS (Mühlmann et al. 2008, Mühlmann and Peintner 2008) with the DOTUR program (Schloss and Handelsman 2005). BLASTn queries of the representative sequence of each OTU were carried out against GenBank and UNITE databases to eliminate chimeric sequences and provide as precise as possible identification of the root-associated fungi, with the taxon identity from UNITE having priority over that from GenBank when there were conflicts. ITS matches for taxa in GenBank were defined as a sequence similarity of 97–100% and e values below e 100 for species, corresponding to at least 90% of the sequence length.

Statistical analyses.—Diversity measures including species richness index (Margalef 1958), evenness (Pielou 1969), Shannon's diversity index (Shannon and Wiener 1949) and Simpson's index of diversity were calculated according to host-plant species and sampling site. Assessments of species richness and distance curve among samples and the overall number of fungal OTUs in relation to sample size were carried out on the whole fungal community of four host-plant species and three sampling sites with the Bray-Curtis distance. Two jackknife estimators of species richness—first and second-order jackknife estimators (Jack1, Jack2), which are sensitive to rare species and small sample size—also were used to evaluate the adequacy of sample size and OTU richness. All estimations were calculated in PC-ORD 5.0.

Fisher's exact test and cluster analyses were performed to detect the influence of host-plant species and sampling site on the composition of the fungal communities. Fisher's exact test was performed with SAS software 9.0, with the frequency of fungal OTUs used as the dependent variable, HOST-PLANT SPECIES and SAMPLING SITE as the independent variables and the significance level set as 0.05. Cluster analysis was conducted with PC-ORD 5.0, with the Sørensen (Bray-Curtis) distance measure and nearest neighbor method. One-way ANOVAs were analyzed using SPSS 19.0, with richness, evenness and diversity as the dependent variables and HOST-PLANT SPECIES or SAMPLING SITE as the independent variables . Significance levels for all tests were $P < 0.05$.

RESULTS

Diversity of root-associated fungi.—A total of 377 sequences were generated from the 60 plant root samples, which were assigned to 154 fungal OTUs (TABLE I, SUPPLEMENTARY TABLE I). Thirty-nine fungal OTUs were detected on the dauciform roots, 20 from *Kobresia capillifolia* and 25 from *Carex parva*, and 22 were unique to this type of root. Reference sequences were found in GenBank and/or UNITE for 36% (56) of the total OTUs, similar taxa in GenBank for ≥ 97%, and 21% (32) recognized at species level. Sixty-four percent (98) of the total OTUs were confirmed as ectomycorrhiza and 4% (6) as endophytes, and 33% (51) were unidentified root-associated fungi. The unidentified OTUs consisted of a few basidiomycetes, including Atheliaceae OTU, *Mycena* OTU and *Astrosporina* OTU, and a number of ascomycetes (TABLE I, SUPPLEMENTARY TABLE I). Eighty-eight (57%) of the total OTUs belonged to basidiomycetes, and 66 (43%) were ascomycetes, assigned to 21 and 37 lineages, respectively (SUPPLEMENTARY FIG. 1). For basidiomycetes OTU richness was high in *Inocybe* (25 OTUs) and *Tomentella/Thelophora* (18 OTUs), followed by *Sebacina* (eight OTUs), *Hebeloma* (six OTUs), *Cortinarius* (four OTUs), *Lactarius* (four OTUs), *Russula* (three OTUs) and *Piloderma* (three OTUs). OTU-rich lineages of ascomycetes included Pezizomycotina (eight OTUs), the *Cenococcum geophilum* complex (seven OTUs), Helotiales (four OTUs), *Alternaria* (three OTUs), *Lachnum* (three OTUs), Leotiomycetes (three OTUs) and *Meliniomyces* (three OTUs) (FIG. 1, SUPPLEMENTARY FIG. 1). The fungal community was comprosed of a few frequent OTUs and a large number of rare OTUs; 20% (31) of the fungal OTUs had a frequency ≥ 3, whereas 96 OTUs (62%) were detected as singletons (SUPPLEMENTARY TABLE I). Although the ectomycorrhizal OTUs were among the most abundant OTUs, many of the most dominant OTUs were root-associated species that are not considered ectomycorrhizal (FIG. 2).

Neither species richness nor the distance curve approached an asymptote with increasing sample size (SUPPLEMENTARY FIG. 2). Therefore a sample size that would produce consistent OTU richness and OTU composition was not reached. The total number of OTUs based on all samples was estimated to be 242 and 297 by the Jack1 and Jack2 estimators, respectively, revealing that our actual sampling recovered only 64% and 52%, respectively, of the corresponding estimated OTUs. Most of the rare OTUs, especially the root-associated community, remained below the detection limit, indicating a need for enhanced sampling.

Diversity and distribution of fungal communities by host-plant species and sampling site.—For the three sampling sites, OTU richness was high in *Inocybe*, *Sebacina* and Helotiales in the Hong Mountains, high in *Inocybe*, *Tomentella*, *Cenococcum* and *Alternaria* in the Daxue Mountains and high in *Inocybe*, *Tomentella*, *Cenococcum* and *Alternaria* in the Baimaxue Mountains. Most of the OTUs at each sampling site were shared by two or more host-plant species and OTU richness was high in *Inocybe*, *Cenococcum* and *Alternaria* (TABLE II).

For the four host-plant species OTU richness was high in *Innocybe, Tomentella* and *Cenococcum* on *Kobresia capillifolia*, high in *Inocybe, Cenococcum* and *Alternaria* on *Carex parva,* high in *Inocybe*, *Tomentella, Cenococcum, Sebacina* and Helotiales on *Polygonum macrophyllum* and high in *Inocybe* and *Alternaria* on *Potentilla fallens.* A number of OTUs were shared by two or more sampling sites, and OTU richness was high in *Inocybe, Tomentella, Cenococcum* and *Alternaria* (TABLE II).

Several general mycobiont lineages and OTUs were shared by different host-plant species and sampling sites in terms of OTU richness and frequency. At a general level, *Inocybe* displayed high OTU richness across the four hosts and three sites. At the species level, 10 OTUs were recovered on all four of the host-plant species collected from the three sampling sites; they included two *Cenococcum geophilum* OTUs, three *Alternaria* OTUs and one OTU each in *Lactarius*, *Lachnum*, *Articulospora*, *Lecythophora* and *Phialocephala* (TABLES I, II). Diversity measures by host-plant species and by sampling site are illustrated (TABLE III).

Fisher's exact test revealed a statistically significant difference in the composition of fungal communities among the four host plants in the Daxue Mountains (P = 1.651E-04, < 0.05) and Hong Mountains (P = 0.0131, < 0.05) but not in the Baimaxue Mountains (P = 0.1093, > 0.05). Statistically significant differences in the composition of fungal communities among the three sampling sites were found in *Carex parva, Kobresia capillifolia* and *Polygonum macrophyllum* (P = 2.065E-04, 2.769E-05, 9.56E-10, respectively, < 0.05) whereas a difference in *Potentilla fallens* was not supported statistically (P = 0.1606, > 0.05). Cluster analyses revealed that samples from the Hong Mountains grouped together, among which samples of *Kobresia capillifolia* and *Carex parva* assembled with high similarity and *Carex parva* and *Polygonum macrophyllum* samples from the Daxue Mountains grouped completely (SUPPLEMENTARY FIG. 3). One-way ANOVAs detected a statistically significant difference only in the Diversity (D) between Daxue Mountains and Baimaxue Mountains (F = 2.688, P = 0.050). These results suggest low host and sampling site specificity but high host and sampling site preference of root-associated fungal community.

TABLE I. Fungal OTUs of mycobionts on four host-plant species collected from three sampling sites. OTU richness within detected fungal lineages and frequency of OTUs by host-plant species and by sampling site are shown. Frequency by host-plant species includes samples collected from three sampling sites, and frequency by sampling site includes samples of four host-plant species

		Frequency						
		By host-plant				By sampling site		
Fungal OTUs	OTU richness	Cp	Kc	Pm	Pf	H	D	B
Basidiomycetes	90							
Hymenogaster	1	0	2	3	1	5	0	1
Hebeloma	6	2	0	4	0	1	3	2
Piloderma	3	0	1	2	0	1	0	2
Hygrocybe	2	2	0	0	0	0	0	2
Russula	3	0	0	2	3	3	1	1
Inocybe	25	**14**	**17**	15	5	21	15	15
Boletales	1	**2**	0	0	0	1	0	1
Amanita	1	0	0	4	0	1	0	3
Clavulina	1	1	1	1	1	0	1	3
Astrosporina	1	0	1	0	0	0	1	0
Laccaria	2	3	0	1	0	1	2	1
Cantharellales	1	2	0	0	0	0	2	0
Ceratobasidium	2	0	0	0	2	0	2	0
Mycena	1	0	0	0	1	1	0	0
Atheliaceae.	1	1	0	0	0	0	0	1
Agaricales	2	0	1	1	0	0	0	2
Cortinarius	4	1	0	3	0	1	2	1
Sebacina	8	0	3	9	0	5	3	4
Tomentella	18	**2**	**11**	10	3	4	11	11
Lactarius	4	1	**6**	4	3	9	1	4
Tremellales	1	0	**1**	0	0	0	1	0
Ascomycetes	66							
Leohumicola	1	0	0	1	0	0	0	1
Leptodontidium	2	**1**	1	1	0	1	2	0
Alatospora	1	0	0	0	1	0	0	1
Pezizomycotina	8	**2**	**7**	2	3	3	6	5
Magnaporthaceae	1	0	0	1	0	1	0	0
Podospora	1	**1**	0	0	0	0	0	1
Phialea	2	**1**	0	1	0	1	1	0
Monodictys arctica	1	0	0	2	0	0	0	2
Leotiomycetes	3	**1**	0	1	1	2	0	1
Microglossum	1	1	0	1	1	0	1	1
*Fusarium**	1	1	1	1	0	2	1	0
Simplicillium	1	0	1	0	0	0	1	0
Geoglossum fallax	1	**1**	0	0	0	0	1	0
Herpotrichiellaceae	1	**2**	0	0	0	0	0	2
Cladophialophora	1	0	0	3	0	3	0	0
Cyphellophora	1	0	**1**	0	0	1	0	0
Exophiala	2	0	**1**	1	0	1	0	1
Cladosporium	1	0	0	1	0	1	0	0

TABLE I. Continued

Fungal OTUs	OTU richness	Frequency						
		By host-plant				By sampling site		
		Cp	Kc	Pm	Pf	H	D	B
Coccomyces	1	0	**1**	0	0	0	0	1
Lecythophora	1	2	2	2	1	3	1	3
*Alternaria**	3	**14**	**17**	11	10	21	13	18
Lophiostoma	1	**1**	0	0	0	0	1	0
Ochrocladosporium	1	0	1	0	1	1	1	0
Rhizoscyphus	1	0	0	0	1	0	1	0
Helotiales	4	0	0	3	2	3	0	2
Protoventuria	1	0	1	0	1	1	1	0
Geomyces	1	1	0	0	0	0	0	1
Cadophora	1	0	**1**	0	0	1	0	0
Chalara	1	0	0	1	0	1	0	0
*Cryptosporiopsis ericae**	1	1	0	1	0	1	1	0
Hyphodiscus	1	**1**	1	1	1	2	2	0
Meliniomyces	3	2	**1**	3	1	1	3	3
Articulospora	3	**5**	**6**	2	3	10	4	2
Phialocephala*	1	4	1	6	3	2	7	5
Lachnum	3	**4**	**2**	3	1	2	5	3
Cenococcum	7	**13**	**12**	23	6	10	24	20
Belonopsis	1	0	1	0	0	0	1	0

Abbreviations: Kc = *Kobresia capillifolia,* Cp = *Carex parva,* Pm = *Polygonum macrophyllum,* Pf = *Potentilla fallens,* H = Hong Mountains, D = Daxue Mountains, B = Baimaxue Mountains.

Notes: Lineages of ectomycorrhizal OTUs are shown by codes in boldface; those of root endophyte OTUs are denoted by codes with asterisk, and the others are unidentified root-associated fungi. Frequency numbers (boldface) include fungal individuals isolated from dauciform roots of *Kobresia capillifolia* and *Carex parva.*

DISCUSSION

Diversity and community composition of ectomycorrhizal fungi.—Concurring with studies on the rhizosphere fungi of alpine plants (Gardes and Dahlberg 1996, Bjorbækmo et al. 2010, Gao and Yang 2010, Ryberg et al. 2011), this study confirms that alpine plants are rich in root-associated fungi. A wide variety of fungal OTUs were detected on the four plants. Basidiomycete OTUs in genera *Inocybe* and *Tomentella* were the dominant ectomycorrhizal taxa in the region, which matches with commonly found species-rich ectomycorrhizal groups found in other alpine areas (Mühlmann and Peintner 2008, Mühlmann et al. 2008, Gao and Yang 2010). With the exception of *Cenococcum geophilum* OTUs, ascomycete ectomycorrhizal fungal OTUs such as *Cadophora* OTU, *Meliniomyces* OTUs and *Rhizoscyphus ericae,* which are common ascomycete ectomycorrhizal groups (Rinaldi et al. 2008, Münzenberger et al. 2009, Tedersoo et al. 2010), were detected at low frequency. Uncommon ectomycorrhizal fungi OTUs, such as *Ceratobasidium* OTUs, which are seldom reported from alpine sites, also occurred in low OTU richness and frequency.

The wide distribution of arctic ectomycorrhizal fungi throughout the arctic and alpine habitats and a variety of habitats both within and beyond the arctic/alpine areas suggest widespread dispersal and wide ecological amplitudes of fungal species in these areas (Timing et al. 2012; 2014). However, more than one-half of the mycobiont OTUs detected in our study are distinct from known fungal taxa in other regions based on BLAST matching, which is consistent with research reports on fungi in the area. Particular geological changes, (i.e. rapid uplift of the Himalayan Mountains, the Qinghai-Tibet Plateau and the Hengduan Mountains and distinctive environmental conditions in the region) promote specific, abundant fungal taxa, which are evolutionarily and ecologically adapted to the environment (Zang et al. 1996).

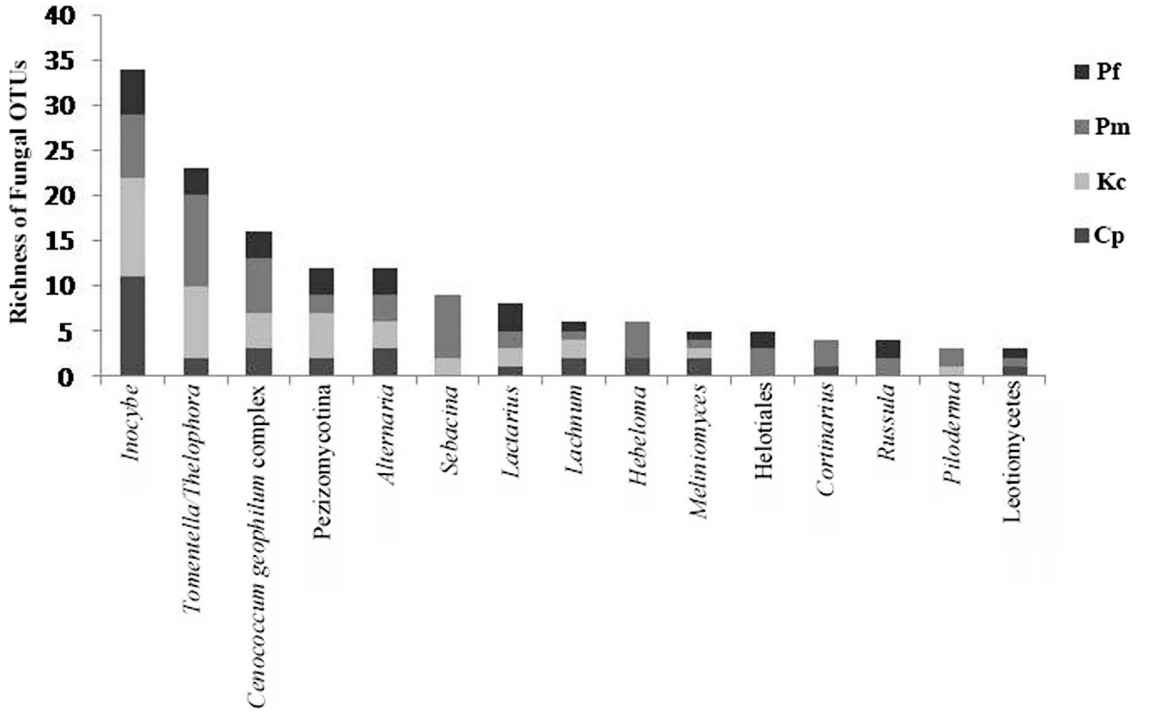

FIG. 1. OTU richness of major root-associated fungal lineages sequenced from four host plant species. Richness based on data from all three sampling sites. Kc = *Kobresia capillifolia*, Cp = *Carex parva*, Pm = *Polygonum macrophyllum*, Pf = *Potentilla fallens*.

Obviously the root-associated fungi detected in this study are only a fraction of the fungal species present in alpine areas in the region and more research on mycobionts associated with alpine plants is necessary to further document the mycoflora inhabiting the region.

Multiple infections on root tips of alpine plants.—Several ascomycete taxa, including *Phialocephala fortinii*, *Alternaria* OTUs, *Cryptosporiopsis ericae* and *Fusarium* OTU, were endophytes on ectomycorrhizal root tips (Rosa et al. 2009), and although not diverse, some were frequent. *Phialocephala fortinii* is a typical general

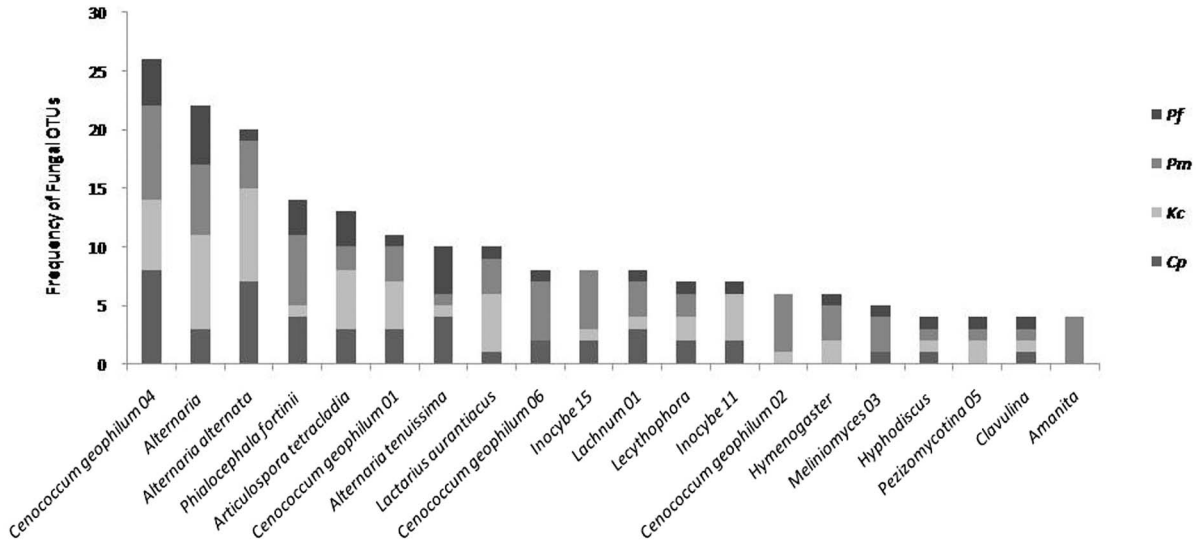

FIG. 2. Frequency of the most frequent fungal OTUs from the four host plant species. Frequency based on data from all three sampling sites. Kc = *Kobresia capillifolia*, Cp = *Carex parva*, Pm = *Polygonum macrophyllum*, Pf = *Potentilla fallens*.

endophyte co-occurring with ectomycorrhizal fungi in alpine regions, which was reported by Rosa et al. (2009) as either an ectomycorrhizal fungus or an endophyte, demonstrating its varied relationship with alpine plants. *Alternaria* species were not dominant endophyte fungi of plants in alpine areas, but three *Alternaria* OTUs were found frequently and were common to the four hosts and three sites in our study.

In addition, a few basidiomycetes, such as *Astrosporina* OTU, *Mycena* OTU, OTU of Atheliaceae and Tremellales and a substantial number of ectomycorrhiza-associated ascomycetes (EAA) (43% of the total fungal OTUs) (Tedersoo et al. 2009), were detected on ectomycorrhizal root tips including the dauciform roots of the alpine plants, indicating multiple colonization on root tips (Wagg and Pautler 2008, Becerra et al. 2009). These root-associated fungi did not belong to general fungal groups in arctic/alpine areas (Timing et al. 2014) and thus were not identified or functionally determined based on DNA sequence data and previous research.

The variety of unnoticed ascomycete OTUs, which were much more diverse than those of basidiomycete taxa, included *Articulospora tetracladia*, *Lachnum* OTU 01 and *Lecythophora* OTU, several generally and frequently detected ectomycorrhiza partners (Mühlmann and Peintner 2008, Mühlmann et al. 2008, Rinaldi et al. 2008, Münzenberger et al. 2009, Tedersoo et al. 2010) and a diversity of infrequent ascomycete groups,such as *Leohumicola* OTU, *Alatospora* OTU and Magnaporthaceae OTU and others, that had not been detected in other regions. These EAA were also an essential part of the root-associated fungi found on alpine plants in the region. They in fact may be dark-septate endophytic fungi or other plant endophytes and some may possibly be parasitic or saprobic opportunists (Tedersoo et al. 2009, Blaalid et al. 2012), indicating specific relationships among fungi, multiple infections on mycorrhizal root tips (Wagg and Pautler 2008, Becerra et al. 2009, Gao and Yang 2010) and a range of potential and variable interactions between fungi and host plants, which may be commensal, neutral, mycorrhizal and/or opportunistic.

Various types of root-associated fungi may exist simultaneously on root tips, and the putatively ectomycorrhiza-forming OTUs of some lineages are closely related to many endophytic taxa that render their identification ambiguous based solely on DNA sequence data. This highlights the need to integrate molecular and morphological-anatomical approaches in research, especially when studying poorly known taxa, to detect various types of root-associated fungi and reduce the possibility of underestimation and misinterpretation.

Distribution of fungal communities in terms of host-plant species and sampling site.—Generalist mycorrhizal fungi can associate with a variety of host-plant species (Gardes and Dahlberg 1996, Timing et al. 2012), whereas the composition of the ectomycorrhizal fungi community has been shown to be affected by host-plant identity (Massicotte et al. 1999, Ishida et al. 2007, Tedersoo et al. 2008). In addition, the structure of the alpine ectomycorrhizal communities also often correlates with environmental factors (Walker et al. 2008, Timing et al. 2012). In our study host and sampling site preference of root-associated fungal community was detected whereas effect of host and site on the composition of root-associated fungi was not statistically significant. This may have been due to insufficient sample size, given that only the 60 plants and three sites were analyzed. Further study is necessary therefore to clarify the distribution of root-associated fungi in terms of host-plant species and sampling sites in alpine areas.

Dauciform roots with mycobionts on Cyperaceae *species.*—Dauciform roots were detected on *Kobresia* spp. and *Carex* sp. in our study, and they were colonized by ectomycorrhiza and/or other mycobionts (Gao and Yang 2010). It should be noted that, unlike the dauciform roots reported in previous research, which are brush-like lateral roots with concentrated root hairs (Shane et al. 2006), those detected on sedges in our research have an exclusively alterative morphology, one of carrot-shaped lateral roots with a smooth surface and no root hairs. The fungal mantle of ectomycorrhiza on the dauciform roots probably hinders the formation of root hairs. Furthermore, because mycobiont infection is functionally similar to root hairs in absorbing nutrients it is possible that the former is a substitute for the latter for sedges in the alpine environment.

Dauciform roots have been observed in several other Cyperaceae plants (e.g. *Caustis blakei*, *Schoenus unispiculatus*) as a response to phosphorus deficiency (Shane et al. 2004). Mycorrhizal colonization in dauciform roots of sedge species also was recorded in drought environments (Meney et al. 1993). Dauciform roots and mycorrhizal roots may mutually reinforce and complement each other in terms of ecophysiological functions in the extreme alpine environment. Therefore the phenomenon of dauciform roots infected by mycobionts on Cyperaceae species appears to be a double adjustment, a reinforced ecophysiological strategy taken by both plants and fungi to survive the challenging environment in alpine regions.

TABLE II. OTU richness within detected fungal lineages by host-plant species and sampling site

	Diversity/host-plant species/sampling site											
	Hong Mountains				Daxue Mountains				Baimaxue Mountains			
Fungal lineages	Kc	Cp	Pm	Pf	Kc	Cp	Pm	Pf	Kc	Cp	Pm	Pf
Total (OTUs/lineages)	25/14	21/16	32/25	15/11	25/18	18/16	24/14	15/9	2614	23/14	32/18	18/14
Hymenogaster	1	0	1	0	0	0	2	0	0	0	0	1
Hebeloma	0	1	0	0	0	0	0	0	0	0	1	0
Piloderma	0	0	0	0	0	0	0	0	0	0	2	0
Hygrocybe	0	0	0	0	0	0	0	0	0	1	0	0
Russula	0	0	0	2	0	0	1	0	0	0	1	0
Inocybe	6	4	4	2	3	1	4	3	6	6	4	0
Boletales	0	0	0	0	0	0	0	0	1	0	0	0
Amanita	0	0	1	0	0	0	0	0	0	0	1	0
Clavulina	0	0	0	0	1	0	0	0	0	1	1	1
Astrosporina	1	0	0	0	1	0	0	0	0	0	0	0
Laccaria	2	1	0	0	0	1	1	0	0	1	0	0
Cantharellales	0	0	0	0	0	1	0	0	0	0	0	0
Ceratobasidium	0	0	0	0	0	0	0	2	0	0	0	0
Mycena	0	0	1	0	0	0	0	0	0	0	0	0
Atheliaceae.	0	0	0	0	0	0	0	0	0	1	0	0
Agaricales	0	0	0	0	0	0	0	0	1	0	1	0
Cortinarius	0	0	1	0	0	0	2	0	0	1	0	0
Sebacina	2	0	3	0	1	0	2	0	0	0	2	0
Tomentella	2	0	1	0	3	2	4	1	4	0	5	2
Lactarius	0	1	2	1	0	0	1	0	1	0	1	1
Tremellales	0	0	0	0	1	0	0	0	0	0	0	0
Leohumicola	0	1	0	0	0	0	0	0	0	0	0	0
Leptodontidium	0	1	1	0	1	0	0	0	0	0	1	0
Alatospora	0	0	0	0	0	0	0	0	0	0	0	1
Pezizomycotina	1	1	1	0	2	1	0	2	2	0	1	1
Magnaporthaceae	0	0	1	0	0	0	0	0	0	0	0	0
Podospora	0	0	0	0	0	0	0	0	0	1	0	0
Phialea	0	1	0	0	0	0	1	0	0	0	0	0
Monodictys	0	0	0	0	0	0	0	0	0	0	1	0
Leotiomycetes	0	1	0	1	0	0	0	0	0	0	1	0
Microglossum	0	0	0	0	0	1	0	0	0	0	0	1
*Fusarium**	0	1	1	0	1	0	0	0	0	0	0	0
Simplicillium	0	0	0	0	1	0	0	0	0	0	0	0
Geoglossum	0	0	0	0	0	1	0	0	0	0	0	0
Herpotrichiellaceae	0	0	0	0	0	0	0	0	1	0	0	0
Cladophialophora	0	0	1	0	0	0	0	0	0	0	0	0
Cyphellophora	1	0	0	0	0	0	0	0	0	0	0	0
Exophiala	1	0	0	0	0	0	0	0	0	0	1	0
Cladosporium	0	0	1	0	0	0	0	0	0	0	0	0
Coccomyces	0	0	0	0	0	0	0	0	1	0	0	0
Lecythophora	0	1	1	0	0	1	0	0	1	0	0	1
Alternaria *	2	2	1	1	2	1	1	3	2	3	2	2

TABLE II. Continued

	Diversity/host-plant species/sampling site											
	Hong Mountains				Daxue Mountains				Baimaxue Mountains			
Fungal lineages	Kc	Cp	Pm	Pf	Kc	Cp	Pm	Pf	Kc	Cp	Pm	Pf
Lophiostoma	0	0	0	0	0	1	0	0	0	0	0	0
Ochrocladosporium	0	0	0	1	1	0	0	0	0	0	0	0
Rhizoscyphus	0	0	0	0	0	0	0	1	0	0	0	0
Helotiales	0	0	3	0	0	0	0	0	0	0	0	2
Protoventuria	0	0	0	1	1	0	0	0	0	0	0	0
Geomyces	0	0	0	0	0	0	0	0	0	1	0	0
Cadophora	1	0	0	0	0	0	0	0	0	0	0	0
Chalara	0	0	1	0	0	0	0	0	0	0	0	0
Cryptosporiopsis	0	0	1	0	1	1	0	0	0	0	0	0
Hyphodiscus	0	0	1	1	1	1	0	0	0	0	0	0
Meliniomyces	0	0	1	0	0	1	1	0	1	1	0	1
Articulospora	2	2	1	2	1	0	0	1	0	2	0	0
Phialocephala	0	0	1	1	0	1	1	1	1	1	1	1
Lachnum	1	1	0	0	0	1	1	0	1	1	0	1
Cenococcum	2	1	1	2	2	2	2	1	3	2	5	2
Belonopsis	0	0	0	0	1	0	0	0	0	0	0	0

Notes: Lineages of ectomycorrhizal OTUs are shown by codes in boldface, and those of root endophyte OTUs are denoted by codes with asterisk.

CONCLUSIONS

The four alpine plants sampled from the mountains of southwestern China were colonized by various types of mycobionts including ectomycorrhizal fungi, endophytes and a high proportion of unidentified root-associated fungi. The high diversity and high endemism of root-associated fungi have important implications for the conservation of biodiversity and ecological preservation in the region. Integration of molecular and morphological-anatomical criteria is needed to identify the heterogeneous root-associated fungi, and continued sampling efforts are needed to reveal the relevant host and environmental distributions of alpine plant mycobionts.

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TABLE III. Diversity measures of fungal assemblages by sampling site and host-plant species. Diversity measures, including richness index (R), evenness (E), Shannon's diversity index (H) and Simpson's diversity index (D), are presented for fungal assemblages of each sampling site for each host-plant species

	Samples/sampling site/host-plant species											
	Hong Mountains				Daxue Mountains				Baimaxue Mountains			
Index	Kc	Cp	Pm	Pf	Kc	Cp	Pm	Pf	Kc	Cp	Pm	Pf
Richness (R)	6.463	6.372	8.358	4.673	6.578	5.533	6.154	5.050	6.750	6.236	8.561	5.774
Evenness (E)	0.942	0.951	0.952	0.955	0.958	0.925	0.918	0.992	0.965	0.975	0.963	0.993
Diversity (H)	3.033	2.895	3.299	2.648	3.043	2.771	2.916	2.686	3.105	3.015	3.368	2.871
Diversity (D)	0.9399	0.9300	0.9546	0.9161	0.9434	0.9136	0.9292	0.9297	0.9486	0.9465	0.9584	0.9418

Abbreviations: Kc = *Kobresia capillifolia,* Cp = *Carex parva,* Pm = *Polygonum macrophyllum,* Pf = *Potentilla fallens.*

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